Alterations in Growth and Chemical Constituents of Tobacco in Response to CO_2 Enrichment[1]

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Tobacco plants were grown in an enriched CO_2 environment from transplant to maturity to examine alterations in growth and chemistry of leaves. Nutrient availability was progressively decreased, and groups of leaves on the main stem were sampled sequentially to approximate culture conditions in the field. Dry weight accumulation increased in all leaf groups at high CO_2 (700 μL L^{-1}), with total canopy leaf weight being increased to 162% of the ambient CO_2 (350 μL L^{-1}) controls. The chemical composition of leaves throughout the canopy was altered substantially. At high CO_2, the concentrations of nonstructural carbohydrates and polyphenols and leaf surface diterpenes and fatty alcohols all were markedly higher, while concentrations of the primary pyridine alkaloids were lower. The results indicate that future increases in atmospheric CO_2 could have a considerable impact on yield and quality of tobacco.

The atmospheric concentration of CO_2 is steadily increasing. Levels of CO_2 have risen from about 250 μL L^{-1} in 1920 to 350 μL L^{-1} at present and likely will exceed 700 μL L^{-1} during the next century (Pearman, 1980; Edwards et al., 1984). Increases in CO_2 concentration will have a significant impact on growth and development of crop plants (Kimball, 1983). The impact probably will be most pronounced with C_3 plants, since CO_2 availability is a primary limiting factor in photosynthesis. In the case of tobacco, previous experiments have indicated that CO_2 enrichment can have a positive effect on growth. Thomas et al. (1975) reported that increasing the CO_2 concentration from 400 to 1000 μL L^{-1} CO_2 for 20 days after transplanting resulted in 27% greater leaf dry weight, while under a high-nutrient regime leaf dry weight was increased 42%. It is unknown to what extent growth would be affected if tobacco plants were exposed to high CO_2 for an entire growth cycle.

In addition to the effects on growth, CO_2 enrichment can cause substantial alterations in the accumulation of assimilates. With tomato (Ho, 1977) and soybean (Huber et al., 1984; Cure et al., 1987) growing in enriched CO_2 conditions, carbohydrate accumulation in leaf tissues was substantially increased. In their experiments with young tobacco plants, Thomas et al. (1975) found starch levels in leaves to be elevated under high-CO_2 conditions. In tobacco such changes are especially important because the carbohydrate concentration in leaves is closely associated with the quality of the consumer product (Tso, 1972). Furthermore, alterations in carbohydrate accumulation likely would be accompanied by changes in the concentration of important secondary compounds within leaves and on the leaf surface, possibly due to dilution effects or altered metabolism.

The available evidence thus suggests that future increases in atmospheric CO_2 could have a considerable impact on yield and quality of tobacco. This investigation was initiated to characterize general changes occurring in leaf growth and chemistry when tobacco plants were grown in an enriched CO_2 environment over an extended time period. The nutrient regime under which plants were grown and leaf samplings were ordered to approximate as closely as possible culture conditions in the field.

EXPERIMENTAL SECTION

Seed of the flue-cured tobacco (*Nicotiana tabacum* L.) cultivar NC 2326 were germinated on Terralite Metro-Mix (Grace Horticultural Products, W. R. Grace Co., Cambridge, MA 02140) in 170-mL plastic pots. Germination and early seedling development took place in a greenhouse at the Crops Research Laboratory, USDA—ARS, Oxford, NC. The seedlings were watered daily (a.m.), received half–strength Hoagland's solution twice weekly, and were exposed to natural sunlight.

After 7 weeks, the plants were transferred to the Duke University phytotron. Twenty-four plants were selected for uniformity and individually transplanted into 25.4-cm diameter, 9-L plastic pots filled with a mixture of vermiculite; gravel, and Turface (1:1:1, v/v/v). The plants were divided randomly and placed in two growth chambers, one with CO_2 maintained at 350 ± 25 μL L^{-1} and the other at 700 ± 50 μL L^{-1}. The CO_2 concentrations were maintained by monitoring with infrared gas analysis and automatic addition of CO_2 (Hellmers and Giles, 1979). Temperature was maintained at 28/22 °C and RH at 70/100% during the 12/12 h light/dark cycle. Irradiance, provided by high-pressure sodium vapor and metal halide lamps, was ca. 1000 μE m^{-2} s^{-1} at plant height at the beginning of the experiment. Plants received nutrients at half-strength Hoagland's solution twice daily (a.m. and p.m.) for 3 weeks until flowering, once daily for the next 2 weeks, and twice weekly for the last 3 weeks of the experiment. At the times when nutrient additions were omitted, plants received deionized water.

There were four harvests for each plant in the two CO_2 treatment conditions. Each plant was used as a replicate in statistical analyses. The first harvest was at flowering, 21 days after transplant (DAT). (The time of flowering was the same in both CO_2 treatments.) The four oldest leaves at the bottom of the main stem (which had yellowed) were harvested (group 1). Also on 21 DAT, the plants were topped (the apical meristem and inflorescence removed). Subsequent leaf harvests occurred at approriate stages of sequential canopy senescence, which were on 35 (four leaves, group 2), 42 (five leaves, group 3), and 49 (remaining uppermost leaves, group 4) DAT. Plants exposed to 350 μL L^{-1} CO_2 had an average of 18 leaves, and those exposed to 700 μL L^{-1} CO_2 had 21 leaves. All lateral

Table I. Effects of Aerial CO₂ Concentration (350 and 700 μL L⁻¹ CO₂) on Dry Weight Accumulation of Cured Tobacco Leaves (LSD Values Apply to CO₂ Treatment Comparisons with Each Leaf Group)

	dry weight			
	g/leaf group		g/leaf	
leaf group	350	700	350	700
1	3.1	6.5	0.8	1.6
2	6.7	16.4	1.7	4.1
3	11.6	24.9	2.3	5.0
4	32.7	39.9	6.5	5.0
whole plant total	54.1	87.7		
LSD 0.05	4.3		0.8	

branches were excised immediately after emergence. Just prior to the second harvest, at 35 DAT, five 2-cm-diameter leaf punches were removed from the leaves 12–14 (from base of stem) of plants in the two treatment conditions for subsequent analysis of leaf surface chemistries. The punches were dipped, individually, eight times in 10 mL of methylene chloride. The surface extract was then frozen on dry ice and stored in a freezer at −80 °C.

Leaf Curing. After each harvest, leaves were placed on ice and within 3 h were subjected to a curing process modified after Johnson (1965). The leaves were suspended in a dark controlled-environment chamber during the yellowing phase. Temperature was increased at 2–4 °C h⁻¹ from 21 to 35 °C, where it was held until leaves had yellowed. Relative humidity was maintained at 85–87%. After 4 days, the yellow leaves were removed and suspended in an oven for drying. The starting temperature of 38 °C was increased, at 4 °C h⁻¹, to 60 °C until the leaves were completely dried. The cured leaves then were weighed and finely ground for later analyses.

Tissue Analyses. The harvested samples from each plant were analyzed separately. A portion of the ground leaf tissue was analyzed for soluble carbohydrates and starch. Tissue was extracted in 80% ethanol at 80 °C. The ethanol insoluble (starch) fraction was separated by centrifugation, resuspended in 1 mL of 0.2 N KOH, and placed in a boiling water bath for 30 min. After cooling, the pH was adjusted to about 5.5 with 200 μL of 1.0 M acetic acid and the gelatinized starch incubated with amyloglucosidase (Sigma Chemical Co. Grade V) at 55 °C for 30 min. Aliquots of the digested starch were analyzed for glucose by an enzyme-linked assay [refer to Kerr et al. (1986)]. The same enzyme assay, supplemented with invertase, was used to quantify soluble carbohydrates in the ethanol-soluble fraction. Ground leaf tissue also was extracted with methanolic KOH and analyzed by gas chromatography for alkaloids (Severson et al., 1981) and with methanol–water for analysis of polyphenols with high-pressure liquid chromatography (Snook and Chortyk, 1982). Leaf surface components in the methylene chloride extracts were analyzed by capillary gas chromatography (Severson et al., 1984, 1985).

RESULTS

Exposure of tobacco plants to enriched CO₂ from transplant to maturity resulted in increased dry weight accumulation throughout the leaf canopy (Table I). Cured leaf weights ranged from 122 to 245% relative to those of the controls exposed to ambient CO₂. The smallest increase occurred in the youngest leaves at the top of the main stem (group 4). Since high-CO₂ plants had a larger number of leaves in group 4 (eight versus five, see methods), the average weight per leaf was lower than that of plants exposed to ambient CO₂, 4.99 compared to 6.54 g leaf⁻¹, respectively.

Table II. Effects of CO₂ Concentration (350 and 700 μL L⁻¹ CO₂) on the Nonstructural Carbohydrate Content of Cured Tobacco Leaves (g/100 g of Tissue) (LSD Values Apply to CO₂ Treatment Comparisons with Each Leaf Group)

leaf group	soluble carbohydrate		starch		total	
	350	700	350	700	350	700
1	14.77	27.23	0.30	3.10	15.07	30.33
2	15.84	30.92	1.16	9.70	17.00	40.62
3	23.56	29.16	1.46	14.80	25.02	43.96
4	19.08	24.70	1.71	14.70	20.79	39.40
LSD 0.05	2.76		2.11		3.81	

Table III. Effects of CO₂ Concentration (350 and 700 μL L⁻¹ CO₂) on the Alkaloid Content of Cured Tobacco Leaves (g/100 g of Tissue × 10) (LSD Values Apply to CO₂ Treatment Comparisons with Each Leaf Group)

leaf group	nicotine		nornicotine		anabasine		anatabine	
	350	700	350	700	350	700	350	700
1	10.32	9.49	0.419	0.138	0.022	0.016	0.178	0.158
2	12.18	8.11	0.389	0.091	0.033	0.017	0.193	0.143
3	16.91	12.11	0.357	0.099	0.068	0.030	0.240	0.175
4	29.60	18.10	0.317	0.118	0.088	0.038	0.298	0.190
LSD 0.05	5.04		0.20		0.021		0.014	

Table IV. Effects of CO₂ Concentration (350 and 700 μL L⁻¹ CO₂) on the Polyphenol Content of Cured Tobacco Leaves (g/100 g of Tissue) (LSD Values Apply to CO₂ Treatment Comparisons with Each Leaf Group)

leaf group	chlorogenic acid		rutin		total polyphenols	
	350	700	350	700	350	700
1	0.74	1.78	0.055	0.104	0.81	1.94
2	1.22	1.99	0.059	0.062	1.29	2.07
3	1.66	1.75	0.059	0.070	1.72	1.83
4	1.11	1.18	0.091	0.100	1.20	1.29
LSD 0.05	0.31		0.012		0.19	

Table V. Effects of CO₂ Enrichment (350 and 700 μL L⁻¹ CO₂) on the Cuticular Chemical Constituents of Tobacco Leaves (μg cm⁻²) (Constituents Were From Leaf Punches Taken from Fully Expanded Leaves 35 DAT; Data Are Presented as Means ± SE)

chemical constituent	350	700
α-ol	0.035 ± 0.001	0.057 ± 0.012
β-ol	0.075 ± 0.007	0.130 ± 0.020
α-diol	18.83 ± 1.64	35.21 ± 4.90
β-diol	6.773 ± 0.534	11.937 ± 1.521
C₂₂-OH	0.233 ± 0.017	0.447 ± 0.042

CO₂ enrichment resulted in increased concentrations of nonstructural carbohydrates in all leaf groups (Table II). Marked increases were apparent in both soluble carbohydrates and starch. In ambient-CO₂ plants, only negligible amounts of starch were detected in the cured leaf tissue (<8% of total nonstructural carbohydrates), while in leaves of high-CO₂ plants starch comprised as much as 37% of the total nonstructural carbohydrates present.

Leaves of high-CO₂ plants had lower concentrations of alkaloids (Table III). Levels of nicotine, nornicotine, anabasine, and anatabine all were decreased considerably. In contrast, CO₂ enrichment increased the concentrations of chlorogenic acid, rutin, and total polyphenols, primarily in the first two leaf groups sampled (Table IV). Similar trends still were evident in groups 3 and 4, but the data were not statistically different.

The concentrations of cuticular constituents of tobacco leaves also were altered by high CO₂ (Table V). The concentrations of the duvane diterpenes [α- and β-

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4,8,13–duvatrien-1-ols (α- and β-ols) and α- and β-4,8,13-duvatriene-1,3-diols (α- and β-diols)] and a representative fatty alcohol (C_{22}-OH) all were increased to a similar extent. The values ranged from 163 to 192% relative to the controls.

DISCUSSION

The results of this experiment indicate that exposure of tobacco plants to enriched CO_2 over an extended time period can cause a marked stimulation in leaf growth. Higher cured leaf weights reflected increases in the weight of individual leaves in the first three groups of leaves sampled and an increased number of leaves in group 4 (Table I). The increases in weight were much greater than the additional increments of accumulated carbohydrate and therefore represented true increases in structural material. The lower weight of individual leaves of high-CO_2 plants at the last sampling likely reflected the fertility regime, with the supply of nutrients being decreased as the experiment progressed. In the field, limited nutrient availability as plants approach vegetative maturation is considered essential for the accumulation of high amounts of nonstructural carbohydrate and secondary products (Weybrew et al., 1974; Long and Weybrew, 1981). Limitations in nutrient availability, however, also restrict leaf growth responses to enriched CO_2 (Sionit et al., 1981; Cure et al., 1988). Even with the diminishing nitrogen supply and the lack of a large growth stimulation at the top of the leaf canopy, the overall enhancement of total canopy dry weight in the enriched CO_2 environment was similar to that observed with other C_3 plants (Cure et al., 1987).

Our results suggest that atmospheric CO_2 enrichment can significantly change the chemical composition of tobacco leaves. The concentrations of carbohydrates, alkaloids, polyphenols, and various leaf surface compounds all have been identified as important chemical components of leaf quality (Long and Weybrew, 1981; Severson et al., 1985), and all were altered substantially by high CO_2. The alterations were not solely related to dilution effects resulting from excessive carbohydrate accumulation. Calculation of total alkaloid content indicates that alkaloid accumulation per plant was similar in control and high-CO_2 plants (1315 and 1286, respectively; refer to Tables I and III). Nevertheless, calculation of alkaloid concentration as a percent of structural dry weight (total DW minus weight of carbohydrates; refer to Tables I–III) reveals that, relative to growth, alkaloid accumulation was decreased ~20% at high CO_2. Furthermore, leaves of high-CO_2 plants tended to have higher concentrations of polyphenol and cuticular constituents despite the carbohydrate dilution effects. The results therefore suggest that significant adjustments occurred in plant metabolism. It is likely that the concentrations of a number of other chemical components of leaf "quality", which were not measured here, were altered as well.

Previous phytotron experiments investigating environmental alterations of tobacco leaf chemistry have been criticized because the chemical composition of control leaves did not resemble that of leaves from field-grown plants (Long and Woltz, 1977). The criticism would seem to be inappropriate in this study. The soluble carbohydrate concentration for the entire leaf canopy of plants at ambient CO_2 (350 mL L^{-1}) was 19.4%, taking into account the different leaf dry weights (Table II), which is similar to the concentration of reducing sugars in leaves of NC 2326 (15.2%) over the years 1984–1986 in official variety tests (Bowman et al., 1984–1986). In addition, the total concentration of alkaloids measured in leaves at ambient CO_2 was 2.72% (Table III), compared to 3.34% in the

official variety tests. Thus, from the relative closeness of the parameters, it would seem reasonable to expect that the types of chemical changes observed also would occur in leaves of field-grown plants growing in an enriched CO_2 atmosphere.

The observed increases in leaf concentrations of polyphenols at high CO_2 (Table IV), especially chlorogenic acid, have important implications concerning the safety of tobacco smoke. Pyrolytic studies on tobacco leaf constituents have shown that chlorogenic acid is the primary precursor of catechol and 4-ethylcatechol (Schlotzhauer and Chortyk, 1981; Schlotzhauer et al., 1982), and catechol is considered a cocarcinogen (Van Duuren et al. 1973). On the basis of pyrolysis data from tobacco with varying chlorogenic acid levels, an increase in chlorogenic acid concentration equivalent to 1% of tissue dry weight could increase smoke catechol by 20–40% (Schlotzhauer et al., 1982).

Alterations in chemical composition such as those observed here also could have implications for the growth response of tobacco in field environments. The CO_2 enrichment treatment resulted in increased concentrations of duvane diterpenes and fatty alcohols on the leaf surface, which have a wide range of biological activities (Severson et al., 1985; Cutler et al., 1986; Jackson et al., 1986). It would be predicted, for example, that higher levels of the duvane diterpenes might lead to increased susceptibility to tobacco budworm moth oviposition but reduced susceptibility to blue mold and other foliar pathogens (Cruickshank et al., 1977). In addition, decreased levels of nicotine, a highly toxic pyridine alkaloid, would lead to further sensitivity to tobacco budworm (Jackson and Severson, 1987). Although such leaf chemistry–pest interactions almost certainly would occur, it is unknown to what extent the plant growth responses to high CO_2 would be modified.

LITERATURE CITED

Bowman, D.; Corbin, T.; Tart, G. *Measured Crop Performance. Tobacco*; North Carolina Agricultural Research Report No. 97; North Carolina State University: Raleigh, NC 1984; p 15.

Bowman, D.; Corbin, T.; Tart, G. *Measured Crop Performance. Tobacco*; North Carolina Agricultural Research Report No. 103; North Carolina State University: Raleigh, NC, 1985; p 17.

Bowman, D.; Kelley, T.; Tart, G. *Measured Crop Performance. Tobacco*; North Carolina Agricultural Research Report No. 107; North Carolina State University: Raleigh, NC, 1986; p 18.

Cruickshank, I. A. M.; Perrin, D. R.; Mandryk, M. Fungitoxicity of duvatrienediols associated with the cuticular wax of tobacco leaves. *Phytopath. Z.* 1977, 90, 243–249.

Cure, J. D.; Rufty, T. W., Jr.; Israel, D. W. Assimilate utilization in the leaf canopy and whole-plant growth of soybean during acclimation to elevated CO_2. *Bot. Gaz. (Chicago)* 1987, 148, 67–72.

Cure, J. D.; Israel, D. W.; Rufty, T. W., Jr. Nitrogen stress effects on growth and seed yield of nonnodulated soybean exposed to elevated carbon dioxide. *Crop Sci.* 1988, 28, 671–677.

Cutler, H. G.; Severson, R. F.; Cole, P. D.; Jackson, D. M.; Johnson, A. W. Secondary metabolites from higher plants: their possible role as biological control agents. *ACS Symp. Ser.* 1986, No. 296, 178–196.

Edwards, J. A.; Reilly, J.; Trabalka, J. R.; Reichle, D. E. *An Analysis of possible future atmospheric retention of fossil fuel CO_2*; DOE Report TROB; NTIS, Department of Commerce, Springfield, VA, 1984.

Hellmers, H.; Giles, L. J. *Carbon dioxide: critique I. Controlled Environment Guidelines for Plant Research*; Tibitts, T. W.,

Kozlowski, t. T., Eds.; Academic Press: New York 1979; pp 229–234.

Ho, L. C. Effects of CO_2 enrichment on the rates of photosynthesis and translocation of tomato leaves. Ann. Appl. Biol. **1977**, 87, 191–200.

Huber, S. C.; Rogers, H. H.; Mowry, F. L. Effects of water stress on photosynthesis and carbon partitioning in soybean plants grown in the field at different CO_2 levels. Plant Physiol. **1984**, 76, 244–249.

Jackson, D. M.; Severson, R. F. Alkaloid development and tobacco budworm survival on isogenic tobacco lines. Proc. Tob. Chem. Res. Conf. **1987**, 41, 45.

Jackson, D. M.; Severson, R. F.; Johnson, A. W.; Herzog, G. A. Effects of cuticular duvane diterpenes from green tobacco leaves on tobacco budworm (Lepidoptera: Noctuidae) oviposition. J. Chem. Ecol. **1986**, 12, 1349–1359.

Johnson, W. H. Influence of harvesting and process variables on bulk curing of bright leaf tobacco. Trans. Am. Soc. Agric. Eng. **1965**, 8, 354–357.

Kerr, P. S.; Israel, D. W.; Huber, S. C.; Rufty, T. W., Jr. Effect of supplemental NO_3^- on plant growth and components of photosynthetic carbon metabolism in soybean. Can. J. Bot. **1986**, 64, 2020–2027.

Kimball, B. A. Carbon dioxide and agricultural yield: An assemblage and analysis of 430 prior observations. Agron. J. **1983**, 75, 779–788.

Long, R. C.; Woltz, W. G. Environmental factors affecting the chemical composition of tobacco. Recent Advances in the Chemical Composition of Tobacco and Tobacco Smoke; Presented at the 173rd National Meeting of the American Chemical Society, New Orleans, LA, 1977; American Chemical Society: Washington, DC, 1977; pp 116–163.

Long, R. C.; Weybrew, J. A. Major chemical changes during senescence and curing. Rec. Adv. Tob. Sci. **1981**, 7, 40–74.

Pearman, G. I. The global carbon cycle and increasing levels of atmospheric carbon dioxide. Carbon Dioxide and Climate: Australian Research; Pearman, G. I., Ed.; Australian Academy of Science: Canberra, 1980; pp 11–20.

Schlotzhauer, W. S.; Chortyk, O. T. Pyrolytic studies on the origin of phenolic compounds in tobacco smoke. Tob. Sci. **1981**, 25, 6–10.

Schlotzhauer, W. S.; Martin, R. M.; Snook, M. E.; Williamson, R. E. Pyrolytic studies on the contribution of tobacco leaf constituents to the formation of smoke catechols. J. Agric. Food Chem. **1982**, 30, 372–374.

Severson, R. F.; McDuffie, K. L.; Arrendale, R. F.; Gwynn, G. R.; Chaplin, J. F.; Johnson, A. W. Rapid method for the analysis of tobacco nicotine alkaloids. J. Chromatogr. **1981**, 211, 111–121.

Severson, R. F.; Arrendale, R. F.; Chortyk, O. T.; Johnson, A. W.; Jackson, D. M.; Gwynn, G. R.; Chaplin, J. F.; Stephenson, M. G. Quantitation of the major cuticular components from green leaf of different tobacco types. J. Agric. Food Chem. **1984**, 32, 566–570.

Severson, R. F.; Johnson, A. W.; Jackson, D. M. Cuticular constituents of tobacco: factors affecting their production and their role in insect and disease resistance and smoke quality. Rec. Adv. Tob. Sci. **1985**, 1, 105–174.

Sionit, N.; Mortensen, D. A.; Strain, B. R.; Hellmers, H. Growth response of wheat to CO_2 enrichment and different levels of mineral nutrition. Agron. J. **1981**, 73, 1023–1027.

Snook, M. E.; Chortyk, O. T. An improved extraction-HPLC method for tobacco polyphenols. Tob. Sci. **1982**, 26, 25–29.

Thomas, J. F.; Raper, C. D., Jr.; Anderson, C. E.; Downs, R. J. Growth of young tobacco plants as affected by carbon dioxide and nutrient variables. Agron. J. **1975**, 67, 685–689.

Tso, T. C. Physiology and Biochemistry of Tobacco Plants; Dowden, Hutchinson and Ross: Stroudsburg, PA, 1972; pp 393.

Van Duuren, B. L.; Katz, C.; Goldschmidt, B. M. Co-carcinogenic agents in tobacco carcinogenisis. J. Natl. Cancer Inst. (U.S.) **1973**, 51, 703.

Weybrew, J. A.; Long, R. C.; Dunn, C. A.; Woltz, W. G. The biochemical regulation of ripening of tobacco leaves. Mechanisms of Regulation of Plant Growth; Bieleski, R. L., Ed.; The Royal Society of New Zealand: Wellington, 1974; pp 843–847.

X-ray Diffraction and Infrared Spectroscopic Studies of Adsorbed Glyphosate

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X-ray diffraction (XRD) and infrared (IR) spectroscopy were used to investigate the bonding mechanism of glyphosate onto clay sized Al^{3+}-, Ca^{2+}-, and Na^+-saturated smectites, kaolinite, hematite, gibbsite, and goethite. Aqueous solutions of glyphosate (0.5%) were buffered at pH 2.0, 4.5, 7.0, or 11.5 and used to treat the clays. Adsorbed glyphosate bands in the infrared spectra of treated smectites were reduced in frequency, indicating a hydrogen-bonding mechanism. X-ray diffraction analysis showed interlayer expansion of both Al^{3+}-saturated smectites treated with glyphosate solutions buffered at pH 2.0. The interlayer spacing of Al^{3+} montmorillonite was also expanded by treatment with glyphosate solutions buffered at pH 4.5. Treated samples of kaolinite, hematite, goethite, and gibbsite exhibited no additional IR bands due to adsorbed glyphosate. Further, X-ray diffraction of glyphosate-treated kaolinite showed no interlayer expansion.

The active ingredient of Roundup, a widely used nonselective herbicide, is the monoisopropylamine salt of glyphosate (Mullison et al., 1979). Glyphosate has been shown to have no herbicidal activity in soils. The speed

of deactivation of glyphosate in soil indicates adsorption to be the primary mechanism of deactivation (Sprankle et al., 1975a). Both minerals and whole soils have both been shown to adsorb glyphosate under a variety of conditions (Hance, 1976; Sprankle et al., 1975b; Nomura and Hilton, 1977).

Sprankle et al. (1975b) also identified the pK_a values for the equilibria of the acid functionalities of glyphosate. The pK_a of cationic glyphosate ($HO_2CCH_2NH_2^+CH_2PO_3H_2$) and neutral glyphosate ($HO_2CCH_2NH_2^+CH_2PO_3H^-$) was

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